

The phantom midge menace: Migratory *Chaoborus* larvae maintain poor ecosystem state in eutrophic inland waters

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ABSTRACT

Chaoborus spp. (phantom midge) are prevalent in eutrophic inland waters. In Lake Soppen, Switzerland, *C. flavicans* larvae diurnally migrate between the methane-rich, oxygen-depleted hypolimnion and sediments, and the methane-poor, oxygen-rich epilimnion. Using a combination of experiments and system modelling, this study demonstrated that the larvae's burrowing activities in and out of the sediment perturbed the sediment and re-introduced sequestered phosphorus into the overlying water at a rate of $0.022 \mu\text{g P ind}^{-1} \text{d}^{-1}$, thereby exacerbating internal nutrient loading in the water column. Fluxes of sediment methane and other reduced solutes enhanced by the larval bioturbation would consume oxygen and sustain the hypoxic/anoxic condition below the thermocline. In addition to increasing diffusive fluxes, migrating larvae also directly transported methane in their gas vesicles from the deep water and release it in the surface water at a rate of $0.99 \text{ nmol CH}_4 \text{ ind}^{-1} \text{d}^{-1}$, potentially contributing to methane emission to air. As nutrient pollution and climate warming persist or worsen in the coming decades, proliferation of *Chaoborus* could intensify this positive feedback loop and delay lake recovery.

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1. Introduction

Eutrophication of inland waters, with symptoms such as high nutrients, excess primary production and deoxygenation of the hypolimnion, is one of the major man-made environmental problems (Smith et al., 2006). Aggressive management practices such as nutrient reduction and artificial aeration often yield very limited success, and the eutrophic conditions can persist for decades or longer (Gächter and Wehrli, 1998; McCrackin et al., 2017). These failures continue to puzzle and frustrate scientists and resource managers (Ibelings et al., 2016). We hypothesize that the successful colonization of inland waters (i.e. lakes) by the phantom midge larvae (*Chaoborus* spp.) drives internal processes that counteract external mitigation efforts and maintains the status quo.

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The prevalence of *Chaoborus* larvae in eutrophic inland waters—with population densities up to $130,000 \text{ ind m}^{-2}$ (Gosselin and Hare, 2003; Sweetman and Smol, 2006)—may be attributed to the larvae's tolerance of low oxygen environments and even toxic hydrogen sulfide that characterize the hypolimnion and sediment in eutrophic lakes. Many *Chaoborus* spp. larvae perform diurnal vertical migration where they reside at depth (i.e. sediment or anoxic deep-water) to avoid planktivorous fish, and ascend to the epilimnion at night to prey on other zooplankton (Dawidowicz et al., 1990; Bezerra-Neto et al., 2012). In tropical lakes, this process may divert energy from planktivorous fish and suppress the production of the latter (Hecky, 1984; Lewis, 1996). Accordingly, much research has been done on the roles of *Chaoborus* spp. larvae in food web dynamics in the water column (e.g. Vanni et al., 1997; Cole et al., 2006).

The instars of *Chaoborus* larvae can exchange gases, including CH_4 (McGinnis et al., 2017; Carey et al., 2018) between their gas vesicles and ambient water, thereby adjusting the gas vesicle volume (Teraguchi, 1975) and gaining considerable energetic

advantage through buoyancy for vertical migration (McGinnis et al., 2017). In eutrophic waters and sediments, the gas typically abundant for this purpose (high partial pressure, low solubility) is methane (CH_4) (McGinnis et al., 2017).

Paleolimnological studies have shown that the appearance of *Chaoborus* remains (e.g. mandibles) in sediments often coincided with a major shift in the lake's trophic state (Sweetman and Smol, 2006; Quinlan and Smol, 2010), but the direct link and feedback between *Chaoborus* colonization and lake eutrophication history remain unclear. To our knowledge, Gosselin and Hare (2003) were the first to observe in the laboratory the bioturbation effects of individual *Chaoborus* larvae as they burrow in and out of the sediment. Based on subsequent experimental and modelling studies, it is hypothesized that this bioturbation activity would release sequestered nutrients and CH_4 into the overlying water at an enhanced rate, thereby pushing the system over an ecological tipping point where the eutrophic state may self-sustain indefinitely (McGinnis et al., 2017; Tang et al., 2017). This hypothesis has not been empirically tested, but would have important implications for lake ecology and management. This study therefore aimed at demonstrating the population-level effect that migratory *Chaoborus* larvae have on nutrient and methane dynamics in lakes.

Lake Soppen (47°5'25"N, 8°4'51"E) is a small eutrophic kettle lake (area 0.26 km², max depth 27 m, mean depth 12 m) in the Canton of Lucerne, Switzerland. Paleolimnological evidence suggests a drastic increase in total phosphorus level in the last decades (Lotter, 2001). Strong bioturbation by *C. flavicans* has caused the destruction of calcareous laminations in the last century in Lake Soppen sediments despite the high calcite deposition rate (Hajdas-Skowronek, 1993). Here we used a combination of experiments and system modelling to demonstrate the quantitative effect exerted by *C. flavicans* larvae on internal nutrient loading in Lake Soppen. In addition, by taking up CH_4 in the porewater and releasing it in the water column, migrating *Chaoborus* larvae are expected to accelerate the upward CH_4 flux over passive diffusion and affect the ambient CH_4 -carbon isotope composition in the epilimnion. We therefore also investigated how *C. flavicans* larvae affect CH_4 transport and isotope composition in Lake Soppen. Taken together, this study provides novel insights into how migratory *Chaoborus* larvae could drive a positive feedback loop between eutrophication, methane transport and deoxygenation in inland waters.

2. Material and methods

2.1. Sediment incubation experiment

Sediment was collected from Lake Soppen by a gravity corer. On shore, the sediment was thoroughly mixed with an electric drill-mixer to standardize the initial conditions among all replicates. Incubations were performed in 12 plexiglass cylinders (diameter 5.6 cm, length 29 cm) with bottom caps. The caps were used to measure out ca. 73 mL of the mixed sediment (ca. 4 cm thick); the cylinders were then pushed into the sediment-containing caps. Surface lake water was gently siphoned into each cylinder, letting the water overflow to replace ca. 3 times its volume to standardize the initial water condition among the replicates. The final volume of the overlying water was ca. 0.5 L. *C. flavicans* larvae (instar 3–4) were collected from ca. 20 m depth, rinsed with lake water and sorted into cups in different numbers (0, 30, 60, 90, 120 and 180, in duplicates), equivalent to an in situ areal density of 0–73,000 ind m⁻². Each cup was randomly assigned to a cylinder and the larvae were gently poured into the cylinder. The cylinders were secured in a basket, which was fastened to pilings and partially submerged in the lake to maintain the temperature. The cylinders remained open

and exposed to the natural day light (Fig. S1).

The incubation lasted from 12th June night until 22nd June 2017 morning (9.5 d total). On the last day, the cylinders were removed one at a time in random order for processing. First, we measured the water temperature and dissolved oxygen (HACH® Portable Multi Meter, model HQ40D); the color appearance of the water was recorded. Afterward, 50 mL of the water was taken from the top with a syringe, and stored refrigerated in an opaque plastic bottle for total phosphorus (TP) (see below). 300–400 mL of the remaining water was gently siphoned, without disturbing the sediment, into a glass bottle to measure CO_2 and CH_4 concentrations and $\delta^{13}\text{C}-\text{CH}_4$ (see below).

2.2. Water column measurements, total phosphorus and dissolved gases

Water column profiles were measured with a Seabird SBE 19plus V2 SeaCAT Profiler CTD (Sea-Bird Scientific, Bellevue, Washington, USA) at a sampling frequency of 4 Hz. The profiler was lowered in the water at $\sim 10 \text{ cm s}^{-1}$ and recorded temperature, pressure, conductivity and dissolved oxygen with about a 3 cm resolution.

Lake water was sampled with a Niskin bottle (5 L) at the deepest point on 22nd May for total phosphorus (TP) and on 13th June 2017 for water column dissolved gases. For TP, water samples were preserved in the cold until measurements. TP was measured spectrophotometrically after potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) digestion (45 min at 130 °C). The same method was used to measure TP in the sediment incubation experiment.

Water for dissolved gases (CH_4 and CO_2) measurements was gently drained from the Niskin bottle into a 1 L glass bottle and let overflow until the volume was replaced 2–3 times (Donis et al., 2017). About half of the water was replaced by atmospheric air. The bottle was immediately capped to create a headspace, then shaken vigorously for at least 2 min to equilibrate the dissolved gases with the headspace. Afterward, the headspace was extracted through a top valve into a gas-sample bag (Supel™ Inert Multi-Layer Foil Gas Sampling Bags) by slowly injecting lake water into the bottom of the bottle through a rubber tubing. CO_2 and CH_4 concentrations and $\delta^{13}\text{C}-\text{CH}_4$ of the gas bag content were measured within one day on a Cavity Ringdown Spectrometer (Picarro G2201-i). Initial concentrations of CO_2 and CH_4 in the sampled water was calculated accounting for initial headspace CH_4 and CO_2 concentrations (before equilibrium assuming atmospheric concentration of 2 and 400 ppmv for CH_4 and CO_2 , respectively), volume ratio (i.e. headspace:water), air and water temperatures, in situ barometric pressure and lake water total alkalinity. The same method was used to measure dissolved gases in the sediment incubation experiment.

2.3. Day-night sampling of *Chaoborus*

Chaoborus flavicans larvae were sampled with an open-close net (0.3 m diameter; 200 μm mesh) through discrete vertical strata: 0–5, 5–10, 10–15, 15–20, 20–25 m (max. depth ca. 27 m). Upon retrieval of the net, the cod-end content was washed into a container. *C. flavicans* larvae were counted on shore immediately afterward. The mesh size was not suitable for capturing the small instars 1–2; hence, only instars 3–4 were counted. Between 13th and 14th June, 2017, sampling was done at sunset (ca. 21:00 h local time), sunrise (ca. 05:00 h) and mid-morning. Between 21st and 22nd June, 2017, we increased the sampling frequency to better capture the nighttime ascent of the larvae.

2.4. Bottle incubation experiments

Surface lake water was aerated for several hours using an aquarium-type air pump to equilibrate its background CH₄ with ambient air. The aerated water was used for washing and for the blank. *C. flavicans* larvae were collected from depths where they were most abundant at the time of the experiment (15–20 m in day; 0–5 m at night). The experiments were done three times (13th June at 16:30, 22nd June at 02:40, 22nd June at 14:30). For the first experiment, the larvae were brought back to shore, rinsed with the aerated lake water, and unknown numbers were added to 120-mL serum bottles. For the latter experiments, the larvae were immediately concentrated on a 1-mm mesh on the boat, briefly rinsed with the aerated lake water, and immediately added to the serum bottles. All serum bottles were topped off with the aerated lake water and crimp sealed. Bottles with only aerated lake water were used as the blank.

The sealed bottles were left overnight to allow the release of CH₄ from *C. flavicans* gas vesicles. Afterward, Synthetic Air (Carbagas: 80% N₂, 20% O₂; ±1%) was injected to create headspace (ca. 50 mL). The bottles were shaken vigorously for ca. 2 min. to equilibrate headspace and dissolved gases. The headspace gas was then displaced into a gas-tight syringe by slowly injecting lake water into the bottle. The gas was then injected immediately into the Picarro spectrometer to measure CH₄ concentration and carbon isotopic signature of CH₄. Total CH₄ was calculated by accounting for dissolved CH₄ in the displaced headspace water. Afterward, the numbers of larvae in the serum bottles were counted.

Stable carbon isotope data are reported in delta notation (δ ‰) relative to VPDB (Vienna Pee Dee Belemnite) following the equation:

$$\delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \cdot 1000$$

with R_{sample} as the ratio of heavy to light C isotope of the sample and R_{standard} the isotope ratio of the Vienna Pee Dee Belemnite standard.

2.5. System modelling of methane and oxygen dynamics

In the sediment incubation experiment, *C. flavicans* bioturbation would increase sediment-water exchange of not only nutrients but also dissolved gases including CH₄. To examine the effect of bioturbation on CH₄ dynamics in the experiment, we apply a system modelling approach by accounting for input (bioturbation, F_{sed} plus gas vesicle transport, R_{chab}) and output (oxidation, R_{ox} plus emission to air, F_{sur}) of CH₄. The mass balance is expressed as

$$\frac{\partial C_{\text{CH}_4}}{\partial t} V = F_{\text{sed}} A - F_{\text{sur}} A + R_{\text{chab}} N_{\text{chab}} - R_{\text{ox}} C_{\text{CH}_4} V \quad (1)$$

where C_{CH_4} is the dissolved methane concentration, V is volume of water in the cylinder, t is time, A is the cross-sectional area of the cylinder. Measurements of CH₄ oxidation rate and Fickian diffusion coefficient (k_{600}) are explained in the supplementary materials. Average CH₄ transport by *C. flavicans* gas vesicles was taken from the bottle incubation experiments (section 2.4).

Assuming the system was at steady state at the end of the experiment (i.e. $\frac{\partial C_{\text{CH}_4}}{\partial t} = 0$; input = output), we then derive the relative CH₄ increase against the control due to *C. flavicans* bioturbation (effective diffusivity). Error associated with the system modelling was assessed with a Monte Carlo analysis using the

standard deviations of specific CH₄ oxidation, *C. flavicans* gas vesicles transport and air-water gas exchange (Suppl. materials).

3. Results

3.1. Water column chemistry

The study was conducted on 12th–22nd June, 2017 when the lake was thermally stratified at 5–9 m (Fig. 1A) with a strong oxycline at 5–6 m separating the oxygen-rich epilimnion and the nearly anoxic water below 6 m (Fig. 1B). Dissolved CH₄ was low (ca. 1 μmol L⁻¹) at the surface and increased with depth in the hypolimnion to a maximum of 700 μmol L⁻¹ (Fig. 1C). The average δ¹³C-CH₄ was -51.0‰ in 0–5 m, and was more negative below the thermocline reaching -65.1‰ within 10–25 m (Fig. 1D). Phosphorus concentration increased by an order of magnitude between the surface (14.9 μg L⁻¹) and the bottom (289.6 μg L⁻¹) (Fig. 1E).

3.2. Chaoborus effects on sediment-water exchange

To test the hypothesis that migrating *C. flavicans* larvae enhance sediment-water exchange of dissolved substances (i.e. phosphorus and CH₄), we conducted an experiment where homogenized lake sediment was incubated in Plexiglas cylinders with surface lake water and different number of larvae (instars 3–4) added. Because the cylinders were uncapped during the experiment, some water (8.96 ± 4.95%) was lost to evaporation. At the end of the experiment, the water was increasingly more turbid and green with increasing number of added larvae (with one exception; Table S1). A fair amount of dissolved oxygen remained (76.1–98.7% sat.) with one exception (38.6% sat.; Table S1). Total phosphorus in the overlaying water increased linearly by a factor of 5 with increasing number of larvae (Fig. 2). The amount of phosphorus added to the overlying water by the larvae (slope of regression line) was 0.21 μg P ind⁻¹ over the course of the experiment (9.5 d), or 0.022 μg P ind⁻¹ d⁻¹.

Because one cylinder was approaching hypoxia, its gas dynamics (e.g. respiration, CH₄ oxidation) were not comparable with the others, and was excluded from further data analysis. Final dissolved CH₄ concentrations increased linearly by a factor of ca. 5 with increasing number of larvae; however, δ¹³C-CH₄ was quite stable at an average of -49.9‰ (Fig. 3A and B). Concurrently, dissolved pCO₂ decreased exponentially (Fig. 3C).

3.3. Diurnal migration of Chaoborus

On 13th–14th June, net sampling showed the integrated density of *C. flavicans* larvae (instars 3–4) was quite consistent throughout the sampling cycle (mean ± sd; 9229 ± 1393 ind m⁻²), but their vertical distribution showed marked diurnal changes (Fig. 4). At 21:00 h, most of them were at 5–15 m (87.5% of the total); at 05:00 h, almost all were concentrated at 5–10 m. As the day progressed, the majority descended to 10–15 m (Fig. 4). The second sampling cycle (21st–22nd June) was conducted at a higher frequency to better capture the nighttime ascent of the larvae. Before sunset, most of the larvae were at 10–20 m. They ascended at sunset (ca. 21:00 h) and occupied the 0–5 m layer at night (between 00:00 h and 02:15 h). The larvae descended again in the morning and reached 15–20 m by mid-day (Fig. 4). The integrated density was more variable than the first sampling cycle; however, the average value (9344 ± 4497 ind m⁻²) was very comparable.

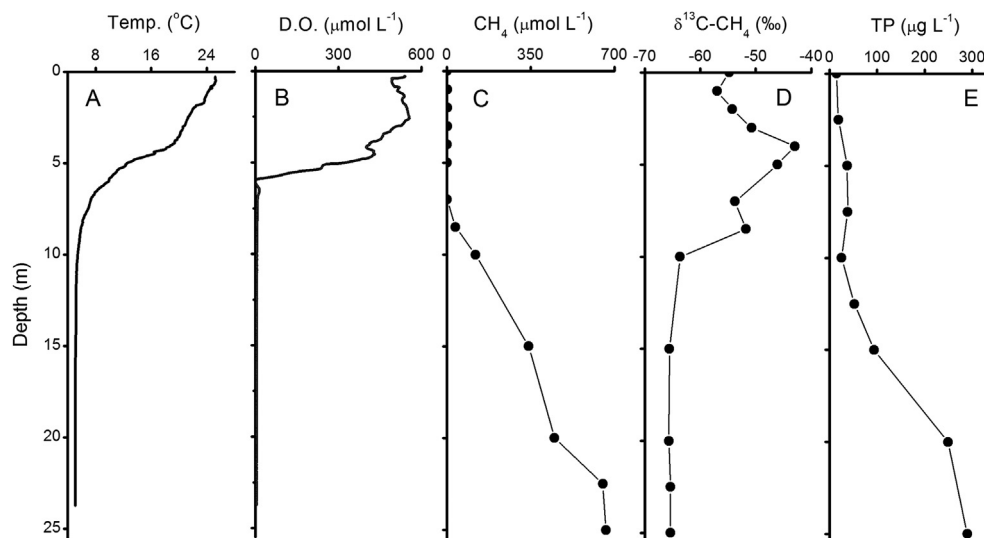


Fig. 1. Water column profiles in Lake Soppen. (A) Water temperature; (B) Dissolved oxygen; (C) Dissolved CH_4 ; (D) $\delta^{13}\text{C}$ of CH_4 ; all measured on 13th June, 2017; (E) Total phosphorus, measured on 22nd May, 2017.

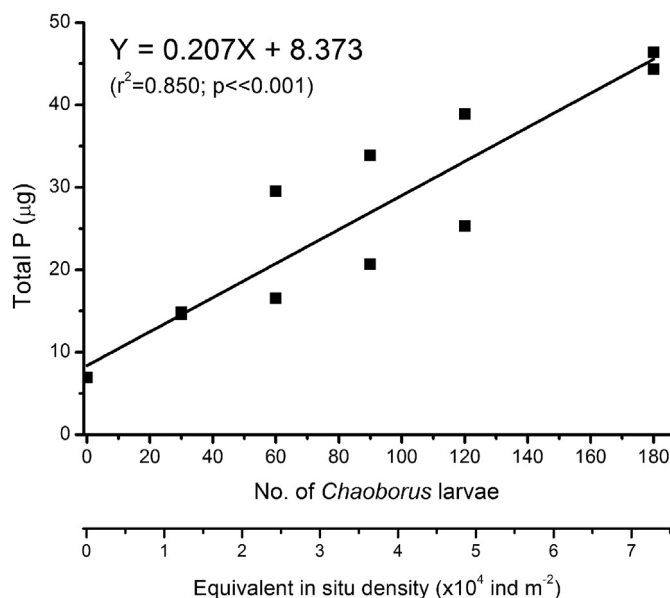


Fig. 2. Final total phosphorus in the water as a function of number of *C. flavicans* larvae in the sediment incubation experiment. The lower x-axis shows the equivalent areal density of the larvae in situ.

3.4. Methane release from Chaoborus gas vesicles

Bottle incubation experiments to measure the release of CH_4 from the larvae's gas vesicles (instars 3–4) were performed three times using larvae collected from the deep (15–20 m) and shallow layers (0–5 m). The total CH_4 released was linearly correlated with number of larvae; i.e. the amount of CH_4 released per individual was quite constant within each trial (Fig. 5A). The amounts of CH_4 released per individual were almost identical between the two trials with deep-water larvae: 0.97 and 1.02 nmol ind^{-1} . Because the shallow-water larvae would have already lost some of their CH_4 to the surrounding water before capture, as expected, the amount of CH_4 was much lower for them, at 0.19 nmol ind^{-1} . Both trials with deep-water larvae gave similar $\delta^{13}\text{C}$ - CH_4 of -65.5 to -62.9‰ , which is very close to that of the bottom water (-65.4‰ ; Fig. 1D).

Shallow-water larvae showed a wider range of values, from -66.15‰ up to -58‰ (Fig. 5B).

3.5. System dynamics of methane and oxygen

The CH_4 oxidation rate was measured to be 0.03 d^{-1} (Table S2). Emission to air was calculated using a measured Fickian diffusion coefficient (k_{600}) of 0.23 m d^{-1} (Suppl. materials) and the final dissolved CH_4 concentrations. Average CH_4 transport by *C. flavicans* gas vesicles was 0.99 $\text{nmol ind}^{-1} \text{d}^{-1}$ (Fig. 5). Under a steady-state condition, our model predicts a linear relative increase in CH_4 as a function of *C. flavicans* abundance (Fig. 6).

The accuracy of our model can be checked by using the phosphorus data as reference for bioturbation influence on diffusivity. In our case, the model outcome for the relative CH_4 increase due to bioturbation (regression slope = 0.035 ind^{-1}) closely matches the observed relative increase in TP (regression slope = 0.032 ind^{-1}); i.e. *C. flavicans* bioturbation increased the effective diffusion of TP and CH_4 by nearly equal magnitude (Fig. 6). The model performance was not sensitive to CH_4 oxidation rate. For example, decreasing and increasing the specific oxidation rate by 10-fold (to 0.003 and 0.3 d^{-1} , respectively) will change the regression slope by $\leq 6\%$. The percent contribution by gas vesicle transport of sediment CH_4 to the overlying water increased with the number of larvae and asymptotically approached 18% of the total CH_4 input (Fig. 6). In other words, the percent contributions of CH_4 by both bioturbation and vesicle transport would approach constant as *C. flavicans* abundance increases, though both rates would presumably continue to increase.

4. Discussion

4.1. Bioturbation and nutrient internal loading

In the sediment incubation experiment, final total phosphorus (TP) concentrations in the control cylinders (without added *C. flavicans*) averaged 15.4 $\mu\text{g L}^{-1}$, almost identical to the in situ surface water value (14.9 $\mu\text{g L}^{-1}$; Fig. 1). In contrast, TP increased at a rate of 0.022 $\mu\text{g P ind}^{-1} \text{d}^{-1}$ in the cylinders with added *C. flavicans* larvae. Because we measured TP (particulate + dissolved), the observed increase in TP cannot be

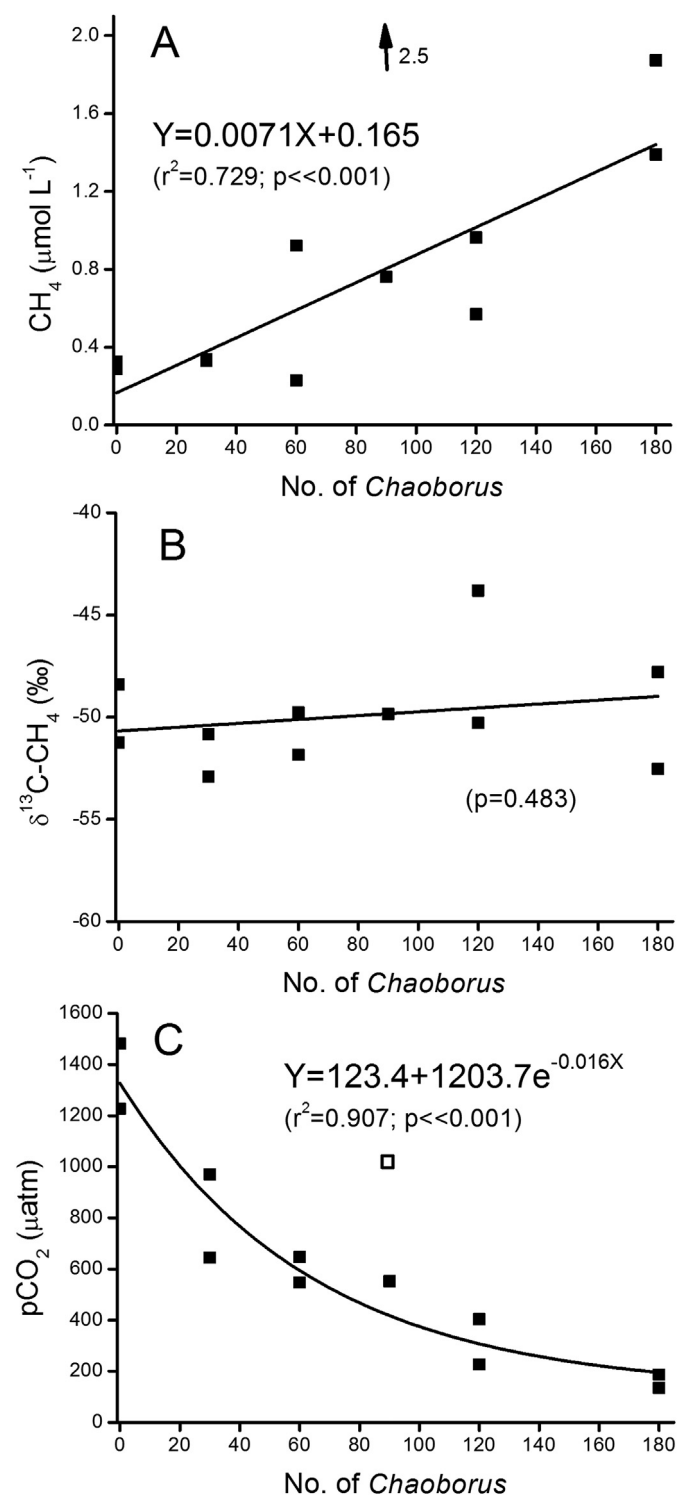


Fig. 3. Water chemistry as a function of number of *C. flavicans* larvae in the sediment incubation experiment. (A) dissolved CH_4 ; (B) $\delta^{13}\text{C-CH}_4$; (C) dissolved pCO_2 . Outliers indicated by arrow and open square were excluded from regression analysis.

attributed to nutrient recycling within the water (e.g. via excretion or remineralization). While we did not count the final number of larvae, we did not observe any dead (floating) larvae in the experiment, which may have contributed to TP via decomposition. To the contrary, live and active larvae were seen in the treatments.

From the data we can infer that bioturbation by *C. flavicans*

increased the effective sediment diffusivity and re-introduced sequestered phosphorus to the overlying water, as has been previously hypothesized (Gosselin and Hare, 2003), and is further supported by our CH_4 data and system modelling. As expected, this extra nutrient stimulated primary production and CO_2 drawdown in the experiment, as indicated by the observed pCO_2 values (Fig. 3C) and the color appearance of the water in the cylinders (Table S1). Extrapolating the experimental results to the observed in situ population density (ca. 9300 ind m^{-2}), *C. flavicans* bioturbation would add ca. 205 $\mu\text{g P m}^{-2} \text{d}^{-1}$ to the water column. This extra phosphorus is likely to first accumulate in the hypolimnion and become available for primary production during spring turn-over. Lake remediation strategies often focus on curbing external phosphorus input (Schindler, 2006). Our results, however, suggest that *C. flavicans* bioturbation is a powerful mechanism to release nutrients from within the sediments, and may explain in some cases the ineffectiveness of external nutrient management for improving water clarity (McCrackin et al., 2017).

4.2. Breaking the diffusive barrier

Oxygen loss from the water column to the sediment is generally limited by diffusion across the sediment-water interface (Bryant et al., 2010). However, hypolimnetic oxygen demand would increase due to increased CH_4 oxidation as a result of enhanced introduction of sediment CH_4 to the overlying water via bioturbation. In the present study, the in situ *C. flavicans* larvae population (9300 ind m^{-2}) was equivalent to 23 larvae added to the incubation cylinder, which according to our model would increase water column CH_4 by 79% over the baseline value via bioturbation alone. The in situ hypolimnetic CH_4 concentrations averaged 445 $\mu\text{mol L}^{-1}$. Assuming a steady state condition, *C. flavicans* bioturbation would increase hypolimnetic CH_4 concentration by 352 $\mu\text{mol L}^{-1}$, and an extra O_2 demand of ca. 703 $\mu\text{mol L}^{-1}$ (or 22 mg L^{-1} ; assuming 1 mol CH_4 : 2 mol O_2 for complete oxidation), more than sufficient to deplete all hypolimnetic O_2 (100% saturation at 12.8 mg L^{-1} at 5 °C). The *Chaoborus*-driven O_2 demand would be even stronger when we consider other reduced solutes and organic carbon re-introduced by bioturbation, plus CH_4 release and respiration by the larvae themselves (Tang et al., 2017). Taken together, *C. flavicans* can greatly increase the hypolimnetic O_2 demand and maintain the hypoxic/anoxic condition below the thermocline, as well as enhance phosphorus loading, which may explain why artificial aeration often fails to restore lake quality (Gächter and Wehrli, 1998).

4.3. A positive feedback loop on climate warming

It is estimated that globally lakes account for 6–16% of natural CH_4 emissions (7–11,300 $\text{mmol m}^{-2} \text{yr}^{-1}$), driven mainly by physical processes such as ebullition and diffusion, whereas the roles of biota, besides a small contribution from rooted vegetation, are largely ignored (Bastviken et al., 2004). In light of our findings, it would be interesting to consider how *Chaoborus* may contribute to CH_4 emission. The enhanced CH_4 flux across the sediment-water interface by bioturbation certainly has immediate implications for hypolimnetic oxygen demand. The added hypolimnetic CH_4 due to *Chaoborus* bioturbation would not be captured by conventional flux measurements at the air-water interface, and the eventual emission of this CH_4 to the atmosphere depends on how fast the lake would overturn, the lake bathymetry (surface area to volume ratio) and oxidation rates. An additional route by which *C. flavicans* can mediate CH_4 flux is by enhancing bubble release through bioturbation (Bezerra-Neto et al., 2012), which is expected to be the

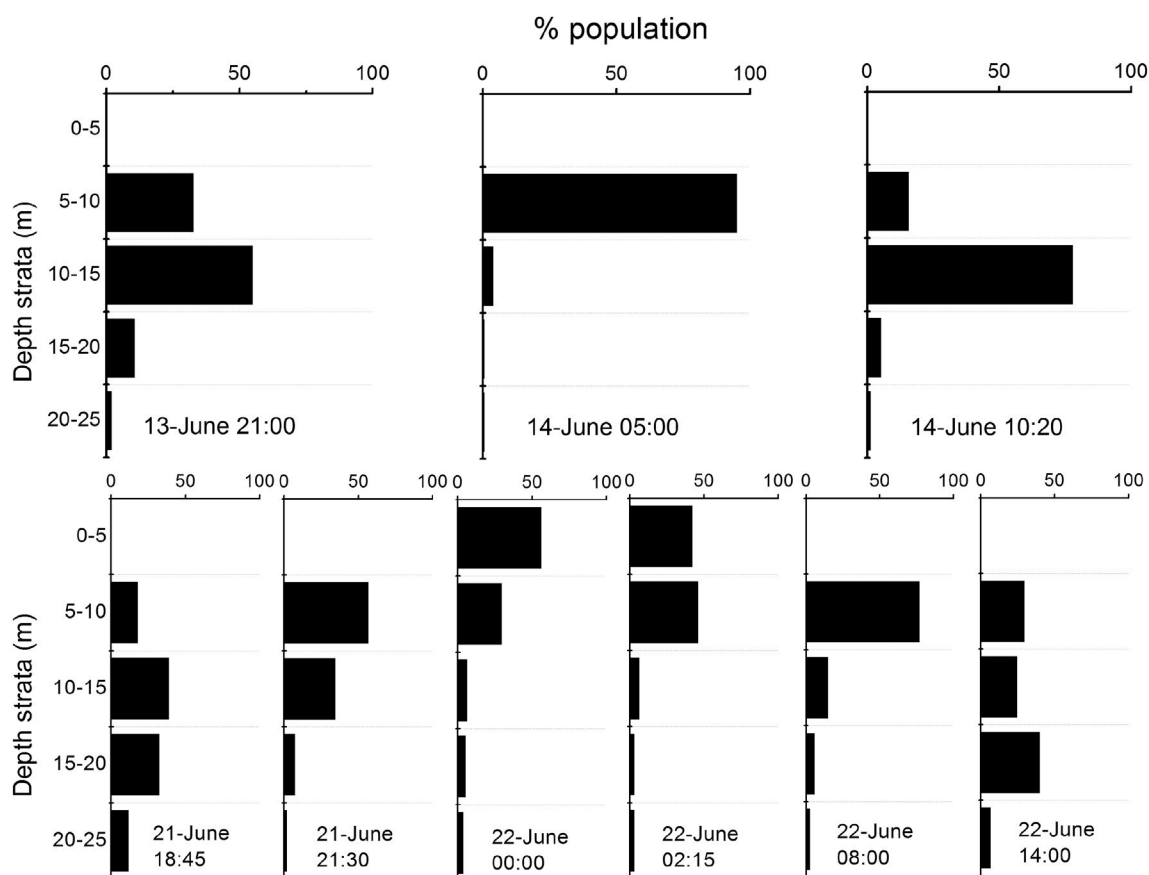


Fig. 4. Percent distribution of *Chaoborus flavicans* larvae (instars 3–4) in the different strata in Lake Soppen at different times on 13th–14th June and 21st–22nd June, 2017.

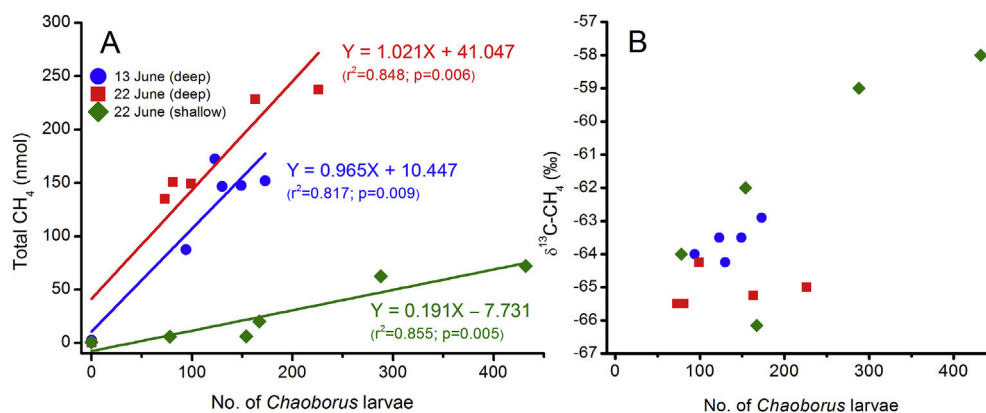


Fig. 5. CH₄ released from gas vesicles of deep- and shallow-water *C. flavicans* larvae (instars 3–4). (A) Total CH₄ as a function of number of *C. flavicans* larvae for deep-water (13th June and 22nd June) and shallow-water (22nd June) samples. (B) δ¹³C-CH₄ of the corresponding samples.

strongest when the larvae perturb the sediment during burrowing (at dawn and at dusk). Likewise, the amount of methane directly transported and released by their gas vesicles to the surface water is expected to be the highest during nighttime upward migration. Both of these processes are unlikely to be resolved by conventional daytime flux measurements.

The observed *C. flavicans* in situ population density (instars 3–4) was ca. 9300 ind m⁻². Based on the bottle incubation experiments with deep-water larvae (averaged 0.99 nmol CH₄ ind⁻¹), we estimate that the amount of deep-water CH₄ transported by gas vesicles would be ca. 9.2 μmol m⁻² d⁻¹. The actual amount would likely

vary in time and in space as the *Chaoborus* population density and activity change. For example, Tang et al. (2017) measured a higher population density of 34,000 ind m⁻² for Lake Soppen in an earlier year. Likewise, McGinnis et al. (2017) estimated that in some lakes, migrating *Chaoborus* larvae may transport up to 2000 mmol CH₄ m⁻² yr⁻¹ from the sediment to the overlying water. It is likely that some of this methane would be lost to oxidation within the water column, and only a small fraction would contribute to emission to air. In a recent study, Carey et al. (2018) estimated that direct transport by migratory *Chaoborus* spp. (mainly *C. punctipennis* in their study) accounted for <1% of the diffusive CH₄ flux to air,

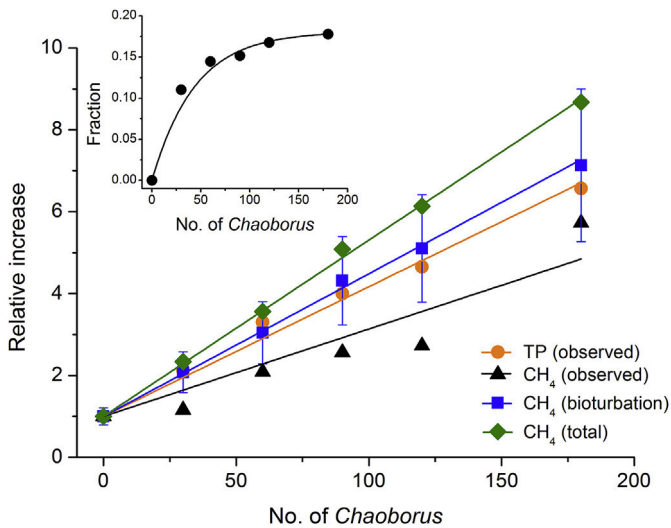


Fig. 6. System modelling results expressed as relative increase against the control, as a function of number of *C. flavicans* larvae. ● = observed TP increase due to bioturbation; ▲ = observed CH₄ increase; ■ = predicted CH₄ increase due to bioturbation; ◆ = predicted total CH₄ increase (bioturbation + gas vesicle transport). Error analysis of the model is explained in supplementary materials. Insert: Fraction of total CH₄ contributed by *C. flavicans* gas vesicles as a function of number of larvae.

although it should be noted that they observed an order of magnitude lower population density than ours.

Eutrophication of inland waters is expected to persist or worsen due to growing human populations and increasing agricultural activities (Tilman et al., 2001; Smith, 2003; McCrackin et al., 2017). Compounding the problem, global warming will exacerbate eutrophication symptoms; for example, rising temperature will increase nutrient remineralization in the catchment and sediment, and thermal stratification will intensify deoxygenation of the bottom layer (Jeppesen et al., 2009; Moss et al., 2011). These conditions are inductive to sediment methanogenesis and favor range expansion and proliferation of *Chaoborus* (Teraguchi, 1975; Taylor et al., 2016). As shown in the present study, bioturbation by *Chaoborus* larvae would significantly increase diffusivity at the sediment-water interface and re-introduce sequestered nutrients, organic carbon and CH₄ to the overlying water. Concurrently, the migrating larvae enhance the direct transport of sediment CH₄ to the surface water for water-air gaseous exchange. By linking the nutrient and CH₄ dynamics in eutrophic lakes, *Chaoborus* can thereby play a unique role in driving a positive feedback loop between lake eutrophication, CH₄ transport and climate warming in the coming decades.

4.4. The *Chaoborus* curse in lake restoration?

The long history of eutrophication has led to a host of public health concerns such as nuisance algal blooms, water quality deterioration and wildlife die-off, causing substantial economic damages (Pretty et al., 2003; Dodds et al., 2008). Studies in eutrophic coastal systems have shown that nutrient reduction often fails to revert the systems to the pre-eutrophication state (Duarte et al., 2009), at least in the short term. Similarly, aggressive lake management and restoration strategies have a checkered record of success (Gächter and Wehrli, 1998; McCrackin et al., 2017). These observations suggest that the systems may have passed the ecological tipping point such that internal forcing tends to maintain the new status quo despite external interventions such as reducing nutrient runoff and artificial aeration. As shown in this and earlier

studies (McGinnis et al., 2017; Tang et al., 2017), *Chaoborus* larvae that have successfully colonized eutrophic inland water bodies drive a positive feedback loop to sustain or even intensify eutrophication and deoxygenation, hindering the system's recovery. We therefore argue that remediation measures will remain limited until sediment CH₄ concentrations drop, and *Chaoborus* abundance is greatly reduced. Understanding this internal *Chaoborus* feedback mechanism may allow resource managers to devise more effective lake restoration strategies.

5. Conclusion

The phantom midge larvae (*Chaoborus* spp.) are prevalent in eutrophic lakes. By virtue of their diurnal migration in and out of the sediment, they significantly perturb the sediment and reintroduce sequestered nutrients and pollutants to the overlying water, essentially maintaining eutrophic lakes in the status quo despite external efforts to curb nutrient inputs and re-oxygenate the water column. Understanding this *Chaoborus*-driven positive feedback mechanism may prove critical in effective lake restoration and in predicting lake ecosystem response to intensifying eutrophication and climate change.

Authors' contributions

KWT, SF and DFM conceived the idea; KWT, SF, DV, CO and DFM performed the study; KWT and DFM analyzed the data; KWT and DFM wrote the manuscript with input from coauthors.

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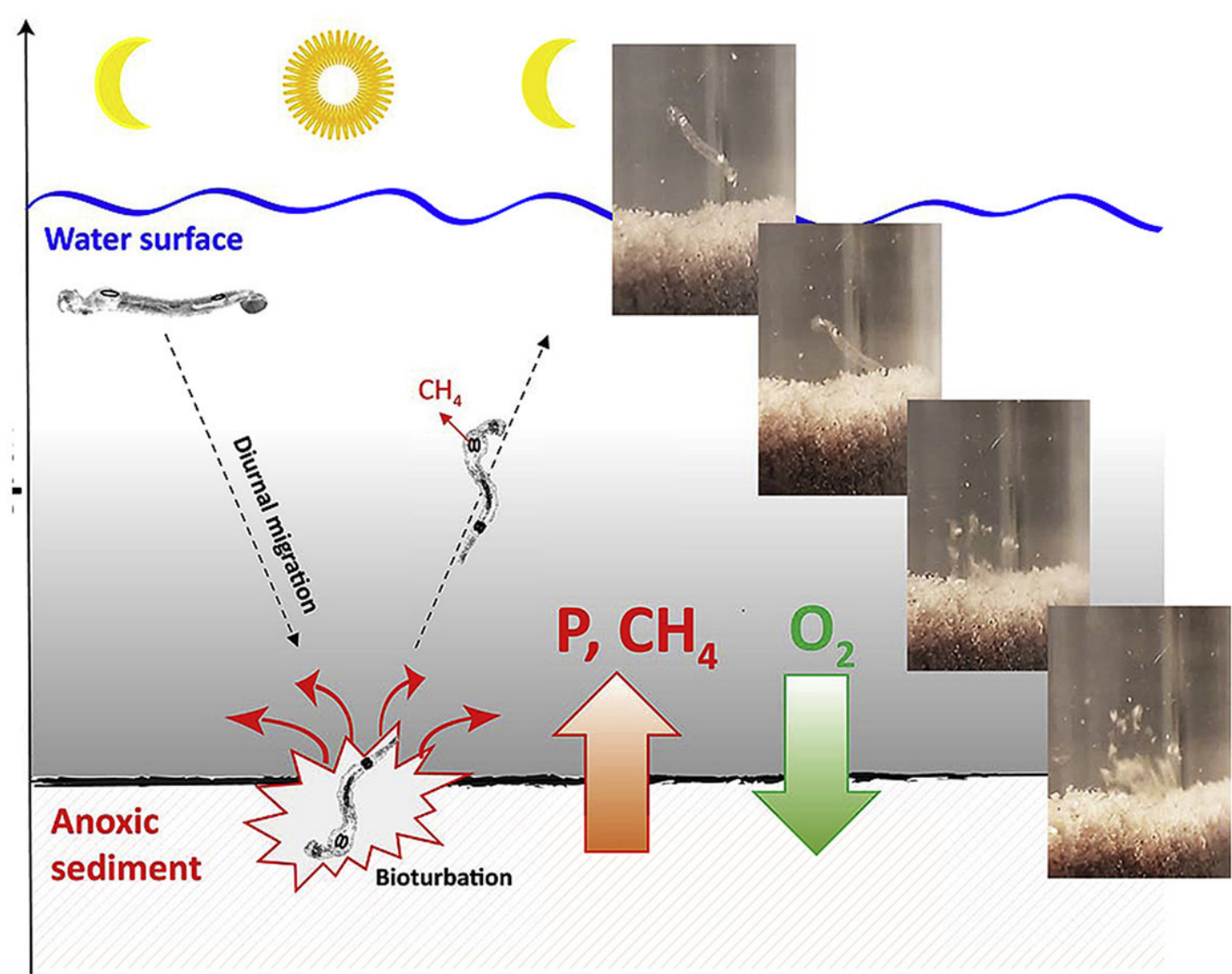
Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.watres.2018.03.060>.

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Supplementary materials

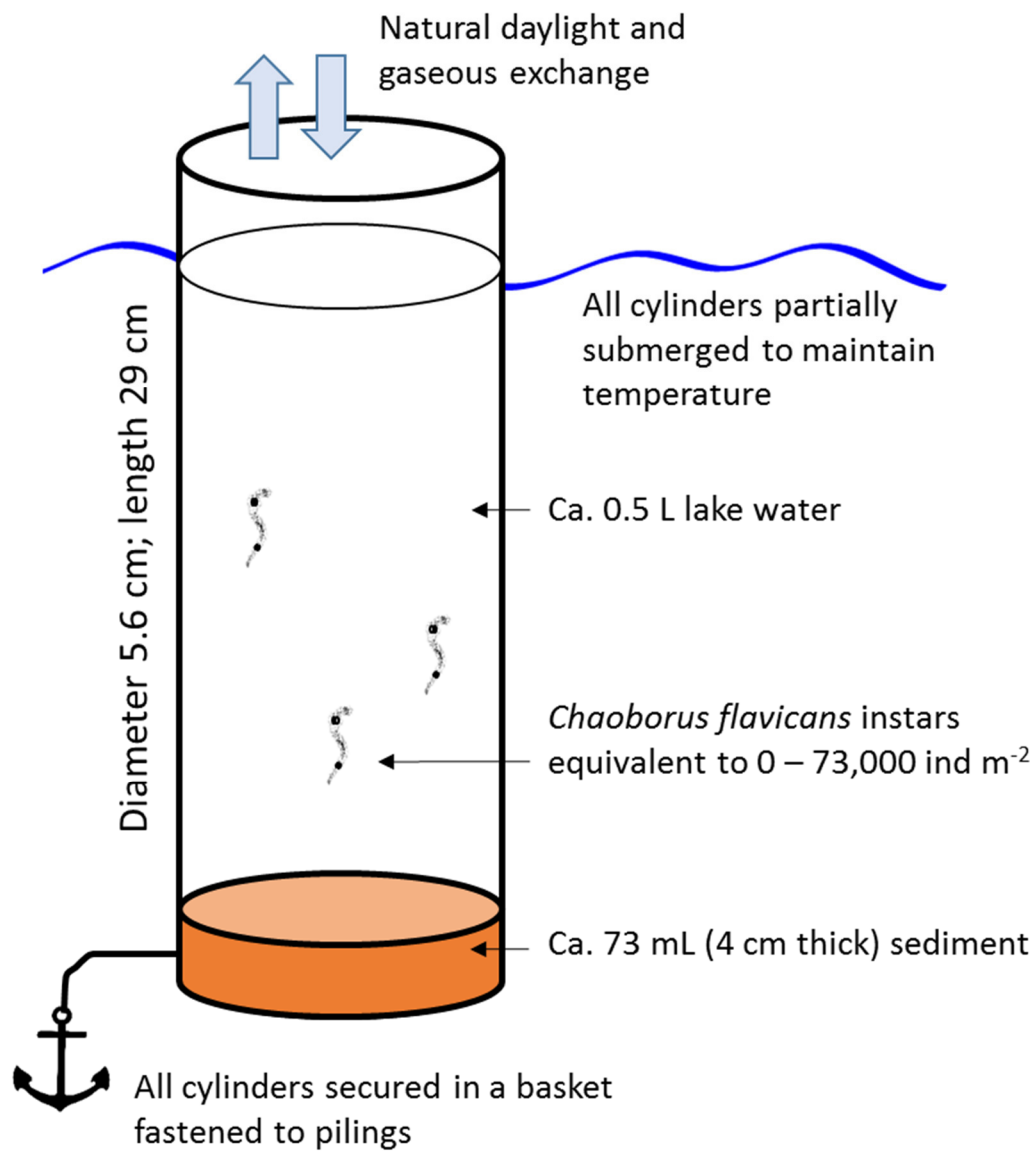


Fig. S1. Schematic of the sediment incubation experimental setup.

Table S1. Water temperature, dissolved oxygen and general appearance of the cylinders at the end of the sediment core incubation experiment.

No. of <i>Chaoborus flavicans</i> larvae	Replicate	Water temp (°C)	DO (mg L ⁻¹)	General appearance
0	A	27.4	6.38	Clear water
	B	27.0	6.09	Very clear water
30	A	28.7	6.12	Water quite clear
	B	26.3	7.02	Water quite clear
60	A	28.1	6.36	Water quite green and turbid
	B	26.6	6.94	Water quite turbid
90	A	27.2	6.57	Almost clear water
	B	28.9	2.97	Yellowish to greenish water color
120	A	26.8	7.93	Green water color; gas bubbles from sediment
	B	26.3	6.82	Water quite green
180	A	27.5	7.80	Very green water
	B	27.6	7.18	Very green water

Methane oxidation experiments

We estimated the CH₄ loss due to oxidation in Lake Soppen using in-situ bottle experiments in summer 2016 (Table S2). On three different dates, we sampled water at three different depths (1, 4 and 7m) using a Niskin sampler and gently poured the water into 120-mL serum glass bottles (with replicates) to avoid degassing. The bottles were incubated in the lake on a mooring line and retrieved on the next visit (see Table S2 for duration). The bottles were

immediately sampled to measure CH₄ concentration by replacing a known volume of water with artificial air (Carbagas: 80% N₂ + 20% O₂). The bottles were shaken vigorously to equilibrate the gases, and the headspace was injected into a Cavity Ringdown Spectrometer (Piccaro G2201-i) to measure CH₄ concentration. Water CH₄ concentration in the bottle was back-calculated accounting for water/air ratio and temperature. CH₄ loss rate was calculated as difference between end concentration in the bottle and in-situ water concentration at the start of incubation (measured in the water column with same method as describe in the main text) divided by the number of days of incubation. Specific oxidation rate (d⁻¹) was calculated as the loss rate (μmol L⁻¹ d⁻¹) divided by the initial concentration (μmol L⁻¹).

Table S2. Methane oxidation from in-situ experiment performed in summer 2016 in Lake Soppen.

Incubation dates		Duration (days)	Specific oxidation rate (d ⁻¹)		
<i>from</i>	<i>to</i>		<i>1m</i>	<i>4m</i>	<i>7m</i>
26.05.2016	15.06.2016	20	0.01	0.02	0.05
15.06.2016	04.07.2016	19	0.01	0.06	0.07
04.07.2016	08.08.2016	35	0.01	0.03	0.03
		mean	0.03		
		s.d.	0.02		

Laboratory estimation of air-water gas exchange

In the laboratory, three cylinders (same dimensions as the ones used for the field experiment) were filled with distilled water and bubbled with N₂ for 30 minutes to significantly reduce the dissolved oxygen. Every hour for 7 hours, dissolved oxygen and temperature were measured at the ca. 2 cm below the air-water interface with an electrode (Unisense, Denmark). Gas exchange coefficients (k_{600} , m d⁻¹) was estimated according to the 1st Fick's law from the linear slope of O₂ increase in the water, accounting for the air-water O₂ gradient and normalized to Schmidt number of 600.

System analysis and error assessment

The system analysis here aimed at estimating the effect of bioturbation on CH₄ release from the sediment to the water. The observed CH₄ in the water at the end of the experiment was the net result of inputs by sediment release (diffusion and bioturbation) and transport by *Chaoborus flavicans* (i.e. by the gas vesicles) and output by CH₄ oxidation in the water and evasion to the atmosphere. Assuming the system was at steady state at the end of the experiment, we can then derive the methane increase due to sediment bioturbation (effective diffusivity) accounting for inputs and outputs. Error associated with the system modelling was assessed with a Monte Carlo (MC) analysis. We used means and standard deviations to estimate normal distributions of rates of specific oxidation, *C. flavicans* transport (vesicles) and k_{600} . Iteratively (n=9999), the MC routine randomly chose the rates according to their distribution to calculate values of CH₄ input from bioturbation. The iterative process then generated a mean value with standard deviation.